Forest Health Protection

Report 04-14 October 2004

PREPLANT SOIL TREATMENT EFFECTS ON PRODUCTION OF BARE ROOT BITTERBRUSH SEEDLINGS LONE PEAK CONSERVATION NURSERY DRAPER, UTAH

R. L. James and S. Zeidler

ABSTRACT

Three preplant soil treatments were evaluated for their effects on the production of bare root bitterbrush seedlings at the Lone Peak Conservation Nursery in Draper, Utah. The two fumigation treatments (standard methyl bromide/chloropicrin [MBC] and chloropicrin alone) reduced levels of soilborne *Fusarium* spp. more than bare fallowing with periodic cultivation. At the end of the growing season, *Fusarium* spp. had reinvaded beds treated with MBC at higher levels than beds treated with chloropicrin alone. Soil fumigation treatments resulted in greater seedling density than bare fallowing. Seedlings from beds treated with chloropicrin had significantly greater heights and diameters at the end of the growing season than seedlings from beds fumigated with MBC. Results of this evaluation indicate that chloropicrin by itself is as effective as MBC in controlling soilborne pathogens, resulting in production of high-quality bitterbrush seedlings. Bare fallowing was not as effective as chemical soil fumigation in reducing pathogen inoculum in soil or controlling seedling disease.

INTRODUCTION

Production of high-quality seedlings for ornamental and reforestation plantings is an important priority of the Lone Peak Nursery in Draper, Utah. Many different hardwood and conifer species are grown annually at the nursery; most of these are produced as bare root seedlings. In order to ensure high seedling quality by reducing damage from soilborne pathogens and competition from weeds, growers at the nursery have routinely implemented preplant soil fumigation with methyl bromide/chloropicrin (MBC)(66% and 33%, respectively) for many years. Although this practice is expensive, it has generally assured predictable production of high-quality seedlings in sufficient numbers to meet demands. However, methyl bromide has been identified as an important chemical destroyer of atmospheric ozone (World Meteorological Association 1995), which limits ultraviolet light from reaching the earth's surface. As a result, methyl bromide is currently being phased out and, for the most part will no longer

United States Department of Agriculture
Forest Service
Northern Region
200 East Broadway
P.O. Box 7669
Missoula, MT 59807

be produced or used in the United States after January 2005 (Shaheen 1996; Stone et al. 1995). Therefore, nursery growers need to develop alternatives to methyl bromide for preplant soil fumigation. Efforts have been widespread throughout the U.S. to develop effective alternatives to methyl bromide in forest nurseries (Chapman 1992; Hildebrand et al. 2004; James et al. 2004a, 2004b, 2004c; Stone et al. 1995). It has generally been found that each nursery is unique in its potential disease problems and suitable alternatives must be designed specifically for that nursery. For example, dazomet, a granular soil fumigant, has been widely used at several nurseries (Boone 1988; Chapman 1992; James et al. 2004a, 2004b). It is usually effective at some nurseries (James et al. 1996; Miller and Norris 1970) but not efficacious at others (James and Beall 1999). Bare fallowing for at least 1 year prior to sowing, accompanied by periodic tilling to keep weed populations low and mix soil is often effective because it leads to reduced organic matter in soil which tends to limit microbial survival (Hildebrand et al. 2004; James and Beall 2000; Stone et al. 1995). Often, potential plant pathogens are reduced in fallowed fields because of the lack of food sources (Hildebrand et al. 2004). When susceptible seedling crops are introduced into fallowed fields, pathogen levels may be too low to initiate substantial disease. The key to effective use of fallowing to control soilborne pathogens is to ensure that supplemental organic matter is not added to soil (Hamm and Hansen 1990; Hansen et al. 1990; Ramirez-Villapudua and Munnecke 1988). Since many potential pathogens are very good saprophytes, they are able to effectively colonize most sources of organic matter, resulting in buildup of populations to the point that they can be damaging to seedling crops (Hamm and Hansen 1990; James et al. 1996).

Bitterbrush (*Purshia tridentata* [Pursh] DC) is one of the most important seedling crops produced at the Lone Peak Nursery. This plant is in high demand for wildlife habitat restoration (Austin and Urness 1983). Seedlings are grown for 1 year in bare root beds; they are then lifted in the fall and kept in cold storage until being shipped to the field, usually the following year. Root diseases, primarily caused by *Fusarium* spp., are major limiting factors in the production of bare root seedlings, including bitterbrush, at the nursery (James 2002a, 2002b). These pathogens initiate a series of diseases including pre- and postemergence damping-off of young germinants and root decay of older seedlings (James et al. 1991, 2004a, 2004b, 2004c). As a result, stands of seedlings may sometimes be greatly reduced and quality of surviving seedlings may be adversely affected (Hildebrand et al. 2004; James et al. 1990, 2004a). *Fusarium* spp. are well-adapted soilborne pathogens that produce resting spores that remain viable for long time periods in the absence of suitable host plants (James et al. 1991; Nelson et al. 1983; Smolinska et al. 2003). Their populations can quickly expand in the presence of organic matter (Hamm and Hansen 1990; Hansen et al. 1990; Ramirez-Villapudua and Munnecke 1988) to levels that can cause diseases on susceptible nursery crops.

Because of the importance of bitterbrush and the prevalence of *Fusarium*-associated diseases at the Lone Peak Nursery, an evaluation was conducted to determine efficacy of different preplant soil treatments on production of bitterbrush seedlings.

MATERIALS AND METHODS

This test was conducted in the production field at the farthest southern end of the nursery. Three treatments were compared: standard operational soil fumigation with MBC at 350 lbs./acre; chloropicin alone at 300 lbs./acre; and bare fallowing with periodic cultivation for one growing season prior to sowing. Preplant treatments were conducted within plots that were 50 feet in length and the equivalent of one seedbed in width (approximately 4-5 feet). Treatments were implemented in a complete randomized block design and replicated four times.

Three sets of soil samples were taken to evaluate effects of soil treatments on populations of *Fusarium, Trichoderma*, a group of common saprophytes and potential antagonists of *Fusarium* (Knudsen and Bin 1990; Knudsen et al. 1991; Papavizas 1985), and *Pythium*, another important group of soilborne pathogens that are known to cause disease problems at some nurseries (James 1982, 1989). The first set was taken prior to treatment to give an overall assessment of resident

populations of these groups of fungi. The second set was taken just prior to sowing in approximately the same locations as the first set to evaluate preplant treatment effects on fungal populations. A final set of soil samples was collected and processed at the end of the growing season to determine fungal population response to presence of the bitterbrush crop.

Samples were collected from within each replicate plot; two samples from near the center of each plot were collected. Each sample consisted of three cores of soil which were mixed together. Each collection consisted of soil cores taken to a depth of about 8 inches (20 cm). Soil was placed in plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard soil dilutions (Hildebrand and Dinkel 1988; James et al. 1990, 1996; Stone et al. 1995) were conducted to estimate populations of *Fusarium, Trichoderma*, and *Pythium* spp. Soil from each sample was initially sieved (2-mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven dried at about 100°C for at least 24 h until sample weight stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For assays of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3% water agar (WA) and thoroughly mixed. One milliliter of solution was placed on each of three plates of selective agar medium for *Fusarium* and closely related fungi (Komada 1975) and spread uniformly. *Trichoderma* propagules were also enumerated on Komada's medium which readily supports growth of this fungus unless the medium is amended with benomyl or lithium chloride (James et al. 1990, 1996). Plates were incubated at least 7 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were expressed as number of colony-forming units (cfu) per gram of oven-dried soil (it was assumed that each fungal colony originated from one propagule). Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for species identification using the taxonomy of Nelson et al. (1983). Ratios of *Trichoderma* to *Fusarium* populations were calculated for each treatment; these ratios may indicate very rough estimates of potential disease suppressiveness of the soil since *Trichoderma* spp. are known antagonists of a wide range of soilborne plant pathogens, including *Fusarium* spp. (Knudsen and Bin 1990; Knudsen et al. 1991; Papavizas 1985).

For assays of *Pythium* populations, 0.5 g of soil was combined with 10 ml of 0.3% WA. One milliliter of solution was placed on each of three plates containing another selective medium consisting of V-8 juice agar amended with the antibiotics pimaricin, rifamycin, and ampicillin and the fungicide pentachloronitrobenzene (James et al. 1990, 1996; Stone et al. 1995). Plates were incubated in the dark at about 24°C for 3 days. *Pythium* colonies were identified on the basis of their diameter after 3 days (15-20 mm), feathery margin, and growth within rather than superficially on the agar surface. Populations were expressed as cfu/g of oven-dried soil.

To determine background levels of potentially pathogenic fungi on bitterbrush seed, two lots [98-310-01 and 00-310-01; 98 and 00 refer to years of collection], which were used to sow the test area, were analyzed. Sampled seed was collected from bulk storage and transferred to the laboratory for analysis. Three hundred seeds from each seedlot were incubated on Komada's medium and emerging fungi identified to genus or species using the taxonomy of Barnett and Hunter (1998) and Booth (1966).

Postemergence disease was evaluated by collecting samples of recently emerged seedlings displaying disease symptoms, such as leaf necrosis. A total of 106 seedlings were sampled from within the three treatment areas. Seedlings were transported to the laboratory and rated for disease severity based on level of above-ground leaf necrosis. Five numerical ratings were used: 1 = no leaf necrosis; 2 = 1%-25% leaf area necrosis; 3 = 26%-50% leaf area necrosis; 4 = 51-75% leaf area necrosis; 5 = greater than 75% leaf area necrosis. Seedling roots were washed thoroughly and dissected into pieces about 5 mm in length. Four root pieces per seedling were randomly selected, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite),

rinsed in sterile, distilled water, and placed on the *Fusarium* selective agar medium. Plates were incubated and emerging *Fusarium* spp. identified as described previously. Percent of sampled seedlings and extent of seedling root colonization by particular *Fusarium* spp. was calculated.

At the end of the growing season (November), seedling density within each replicated block for each treatment was measured and expressed as number of seedlings per square meter. Means for each treatment were compared with a one-way analysis of variance. Significant differences among the means were located with the LSD statistical test. After density measurements, fifty "average" seedlings from each of the three treatment areas were collected for analysis of morphological characters. Seedling heights (from the groundline to the tip the terminal bud on the longest stem), diameter (just above the groundline) and root biomass (oven-dry weight of all roots below the groundline) were measured. Means for each treatment were statistically compared with a one-way analysis of variance and LSD multiple comparison test. Significant differences were expressed at P=0.05.

A final sampling of diseased seedlings was conducted at the end of the growing season. Six seedlings displaying root disease symptoms (leaf necrosis and branch dieback) were collected from within the test area. Seedling roots were washed thoroughly and dissected into pieces about 5 mm in length. Fifteen root pieces per seedling were selected, surface sterilized, rinsed and incubated on the selective *Fusarium* agar medium. Plates were incubated and emerging *Fusarium* spp. identified as described previously. Percent of sampled seedlings and extent of seedling root colonization by particular *Fusarium* spp. was calculated.

RESULTS

Soil in the field selected for this evaluation was populated with relatively high levels of *Fusarium* and corresponding low levels of *Trichoderma* (table 1). The low T/F ratios probably indicated low disease suppressiveness. Populations of *Pythium* were low throughout the field.

At the time of sowing, *Fusarium* populations had been greatly reduced in plots treated with MBC and completely eliminated in chloropicrin-treated plots (table 2). Bare fallowing also reduced *Fusarium* populations, but not nearly as much as chemical fumigation and surviving populations still exceeded expected disease thresholds (Hildebrand and Dinkel 1988; James et al. 1990, 1996). *Trichoderma* levels were likewise greatly reduced by chemical fumigation and either increased or did not change in fallowed plots. *Pythium* populations were eliminated by chemical fumigation and greatly reduced by fallowing.

By the end of the growing season, *Fusarium* levels had increased to much higher levels in MBC-treated plots than in those treated only with chloropicrin (table 3). Fallowed plots also had higher *Fusarium* populations by the end of the growing season. *Trichoderma* levels also increased more in MBC-treated plots compared to chloropicin-treated plots but did not change much in fallowed plots. *Pythium* levels increased much more in fallowed plots but were still at very low levels in fumigated plots.

Six different *Fusarium* species were identified from soil isolates within the test area (table 4). Initially, *F. solani* (Mart.) Appel & Wollenw. was by far the most prominent species; following treatment, *F. solani* and *F. oxysporum* Schlecht. were detected at similar levels. By the end of the growing season, *F. solani, F. oxysporum* and *F. equiseti* (Corda) Sacc. were all isolated at relatively similar levels. *Fusarium chlamydo-sporum* Wollenw. & Reinking was initially detected at higher levels than was found following soil treatments or at the end of the growing season. The other two species, *F. acuminatum* Ell. & Ev. and *F. scirpi* var. *compactum* (Lambotte & Fautr.) Wollenw. were detected at very low levels during each assay.

Table 1. Average pre-treatment soil populations of *Fusarium, Trichoderma* and *Pythium* spp. at the Lone Peak Nursery, Draper, Utah[1].

Treatment	Fungus			
	Fusarium	*Trichoderma*	T/F Ratio[2]	*Pythium*
MBC[3]	2180	324	0.149	21
Chloropicrin[4]	2651	776	0.079	35
Fallow - 1	2585	254	0.098	30
Fallow - 2	2901	481	0.166	28
All Treatments	2579	460	0.178	28

[1]Values in table are colony-forming units per gram of oven-dry soil.
[2]Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote more potential for disease suppressiveness.
[3]Methyl bromide-chloropicrin applied at 350 lbs./acre.
[4]Applied at 300 lbs./acre.

Table 2. Average pre-sowing soil populations of *Fusarium, Trichoderma* and *Pythium* spp. at the Lone Peak Nursery, Draper, Utah[1].

Treatment	Fungus			
	Fusarium	*Trichoderma*	T/F Ratio[2]	*Pythium*
MBC[3]	67	126	0.125	0
Chloropicrin[4]	0	8	0	0
Fallow - 1	1486	667	0.593	3
Fallow - 2	1612	465	0.287	7
All Treatments	791	317	0.399	2

[1]Values in table are colony-forming units per gram of oven-dry soil.
[2]Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote more potential for disease suppressiveness.
[3]Methyl bromide-chloropicrin applied at 350 lbs./acre.
[4]Applied at 300 lbs./acre.

Table 3. Average end-of-first-growing-season populations of *Fusarium, Trichoderma* and *Pythium* spp. at the Lone Peak Nursery, Draper, Utah[1].

Treatment	Fungus			
	Fusarium	*Trichoderma*	T/F Ratio[2]	*Pythium*
MBC[3]	605	485	0.802	19
Chloropicrin[4]	108	149	1.378	9
Fallow	2163	498	0.2302	149
All Treatments	1159	397	0.343	74

[1]Values in table are colony-forming units per gram of oven-dry soil.
[2]Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote more potential for disease suppressiveness.
[3]Methyl bromide-chloropicrin applied at 350 lbs./acre.
[4]Applied at 300 lbs./acre.

Table 4. *Fusarium* species isolated from soil samples – Lone Peak Nursery, Draper, Utah.

Fusarium Species	Sample Period[1]		
	Pretreatment	Presowing	End of Growth
F. solani	72.8	45.8	39.4
F. oxysporum	10.8	46.3	32.1
F. equiseti	1.6	4.7	25.1
F. chlamydosporum	12.3	1.6	0
F. acuminatum	2.5	1.6	1.9
F. scirpi/compactum	0.1	0	1.6

[1]Values in table are percent of *Fusarium* isolates for particular species; sample isolate totals: pretreatment [1207]; pre-sowing [384]; end of growth [315]; total isolates examined [1906].

Fungal colonization of the two assayed bitterbrush seedlots is summarized in table 5. *Penicillium* spp. were by far the most common fungal contaminant; *Trichoderma* spp. were detected at much lower levels on seeds. *Botrytis cinerea* Pers.:Fr., which may be an important foliar pathogen of many different plants (James 1984) was also detected on both seedlots. The other fungus detected, *Cylindrocarpon didymium* (Hartig) Wollenw., was found only on seedlot 00-310-01.

The most frequently isolated *Fusarium* species from recently-emerged diseased seedlings was *F. solani* (table 6). This was predictable because of the high levels of this species occurring in soil (table 4). The other four species isolated from diseased seedlings (*F. equiseti, F. acuminatum, F. oxysporum*) were also detected in soil but were found on seedling roots at much lower levels than *F. solani*.

Table 5. Fungal colonization of bitterbrush seed – Lone Peak Nursery, Draper, Utah[1].

Fungi	Seedlot	
	98-310-01	00-310-01
Penicillium spp.	100.0	99.0
Trichoderma spp.	8.7	40.0
Botrytis cinerea	3.3	8.7
Cylindrocarpon didymium	0	2.7

[1]Figures in table are percent of seed colonized by particular fungi; 300 seeds sampled per seedlot.

Table 6. Infection and colonization of recently-emerged diseased bitterbrush seedlings by *Fusarium* species at the Lone Peak Nursery, Draper, Utah.

Treatment	Number Sampled[1]	Disease Rating[2]	*Fusarium* Species (Percent)[3]			
			FSOL	FEQU	FACU	FOXY
Infection						
MBC[4]	31	4.3	87.1	9.7	64.5	25.8
Chloro[5]	21	4.0	84.0	4.0	48.0	16.0
Fallow	48	3.5	96.0	14.0	26.0	0
All[6]	106	3.9	90.6	10.4	42.4	11.3
Colonization						
MBC[4]	124	4.3	63.7	3.2	24.2	10.5
Chloro[5]	100	4.0	64.0	2.0	22.0	10.0
Fallow	200	3.5	90.0	7.0	11.5	0
All[6]	424	3.9	76.2	4.7	17.7	5.4

[1]Number of seedlings (infection) and root pieces (colonization) sampled; four root pieces sampled per seedling.
[2]Rating based on extent of above-ground leaf necrosis: 1 = No necrosis; 2 = 1-25% leaf area necrosis; 3 = 26-50% leaf area necrosis; 4 = 51-75% leaf area necrosis; 5 = Greater than 75% leaf area necrosis.
[3]Percent of sampled seedlings and root pieces infected by particular *Fusarium* species. Fungal abbreviations: FSOL = *F. solani*, FEQU = *F. equiseti*, FACU = *F. acuminatum*, FOXY = *F. oxysporum*.
[4]Methyl bromide-chloropicrin applied at 350 lbs./acre.
[5]Applied at 300 lbs./acre.
[6]All treatments.

Significantly higher seedling densities at the end of the growing season were detected within plots fumigated with either MBC or chloropicrin alone than in fallowed plots (table 7). No significant differences were found in seedling density between plots fumigated with MBC or chloropicrin alone.

Average seedling height and diameter at the end of the growing season was significantly greater in chloropicrin-treated and fallowed plots than in plots fumigated with MBC (table 8). No differences among the three treatments were detected for root biomass. The greater seedling heights and diameters detected on seedlings from fallowed plots may have resulted from lower seedbed densities occurring in these plots; lower densities would mean more growing room for surviving seedlings and thus larger sizes for these plants (Hildebrand et al. 2004).

By the end of the growing season, roots of diseased seedlings were colonized by three *Fusarium* species (*F. solani, F. equiseti* and *F. acuminatum* - table 9), all of which had been detected on younger seedlings (table 6) or within soil (table 4). *Botrytis cinerea* was also detected on roots of one of the sampled seedling, although at very low levels.

Table 7. Effects of preplant soil treatment on density of bitterbrush seedlings at the end of the growing season at the Lone Peak Nursery, Draper, Utah.

Treatment/Replication	Average Density/m^2	Standard Deviation
MBC – 1	18.3	16.4
MBC – 2	21.3	9.9
MBC – 3	38.8	11.3
MBC – 4	29.5	22.0
All MBC[2]	27.0 A[1]	16.7
Chloropicrin – 1	18.3	7.8
Chloropicrin – 2	36.0	9.6
Chloropicrin – 3	37.3	11.8
Chloropicrin – 4	26.7	7.1
All Chloropicrin[3]	29.6 A[1]	11.7
Fallow – 1	12.0	4.1
Fallow – 2	13.5	8.8
Fallow – 3	11.3	9.5
Fallow – 4	20.8	8.9
All Fallow	14.4 B[1]	8.5

[1]Among treatments, means followed by the same capital letter are not significantly different (P=0/05) using LSD.
[2]Methyl bromide-chloropicrin applied at 350 lbs./acre.
[3]Applied at 300 lbs./acre.

Table 8. Effects of pre-plant soil treatments on height, diameter and root biomass production on 1-0 bitterbrush seedlings – Lone Peak Nursery, Draper, Utah.

Morphological Parameter	Treatment		
	MBC[1]	Chloropicrin[2]	Fallow
Height (cm)			
Mean[3]	31.3 A	35.6 B	35.7 B
Standard Deviation	7.8	6.3	6.2
Standard Error	1.1	0.9	0.9
Minimum	14.0	18.5	25.0
Maximum	54.5	48.5	48.5
Diameter (mm)			
Mean[3]	5.4 A	6.4 B	6.1 B
Standard Deviation	1.9	1.5	1.6
Standard Error	0.3	0.2	0.2
Minimum	1.6	3.9	3.7
Maximum	9.1	10.0	11.3
Root Biomass (g)			
Mean[3]	2.8 A	3.3 A	3.2 A
Standard Deviation	1.5	1.6	1.5
Standard Error	0.2	0.2	0.2
Minimum	0.3	0.7	1.1
Maximum	6.6	8.2	7.7

[1]Methyl bromide-chloropicrin applied at 350 lbs./acre.
[2]Applied at 300 lbs./acre.
[3]Among treatments, means followed by the same capital letter are not significantly different (P=0.05) using LSD.

Table 9. Infection and colonization of diseased bitterbrush seedlings at the end of the growing season by *Fusarium* and *Botrytis* at the Lone Peak Nursery, Draper, Utah.

	Number Sampled[1]	Fungal Species (Percent)[2]			
		F. solani	*F. equiseti*	*F. acuminatum*	*Botrytis*
Infection	6	83.3	83.3	83.3	16.7
Colonization	90	16.7	23.3	12.2	1.1

[1]Number of seedlings (infection) and root pieces (colonization) sampled; fifteen root pieces sampled per seedling.
[2]Percent of sampled seedlings and root pieces infected by particular fungal species.

DISCUSSION

Production of bare root seedlings in forest and conservation nurseries has been plagued with disease problems incited by soilborne pathogens and adverse effects of high weed populations (Boone 1988; James 1989). To overcome these problems, growers implemented preplant soil fumigation with wide spectrum biocides (Boyd 1971; Ibarbia 1995; James 1989). These chemicals nonselectively killed most soil organisms, including populations of potential pathogens (James 1989). Although there are instances of disease control failures following fumigation, primarily due to accelerated reinvasion of treated fields by pathogens (Marois et al. 1983; Vaartaja 1967), in most cases, seedling production is enhanced by treatments (Hansen et al. 1990; James et al. 1990). Resulting seedlings are often larger and appeared healthier than those produced in non-fumigated ground (Boyd 1971; James 1989; James et al. 1990, 1996). In addition, larger numbers of seedlings are usually produced in fumigated fields (Hansen et al. 1990; James 1989). The high cost of soil fumigation is generally more than offset by improved seedling production and quality. As a result, preplant soil fumigation has become routine at many nurseries (Boone 1988; James 1989). Those nurseries that have started the practice usually have to continue fumigation prior to every subsequent seedling crop because high levels of seedling losses may occur if fumigation is stopped (Ibarbia 1995; Linderman et al. 1994; Smith and Vega 1966).

Throughout the history of soil fumigation in agriculture, several different products have been evaluated. Most had some disadvantages, such as selective toxicity to soilborne organisms (Campbell and Kelpsas 1988), penetration problems through soil (Gandy et al. 1976; Kolbezen et al. 1974; Munnecke and Van Gundy 1979), and potential environmental hazards (Linderman et al. 1994; Shaheen 1996). After much experience with other agricultural systems, the fumigant of choice became a mixture of methyl bromide and chloropicrin [MBC] [usually 66% methyl bromide and 33% chloropicrin] in forest and conservation nurseries (Boyd 1971; Cordell 1982; Hildebrand and Dinkel 1988; James 1989). This fumigant, when applied properly, was very effective in controlling both soilborne pathogens and weeds (Boyd 1971; James 1989). As a result, many bare root nurseries instituted applications of MBC, usually prior to sowing each seedling crop. Unfortunately, methyl bromide, the major component of this fumigant, was found to be an important destroyer of atmospheric ozone and thus will not be available for use in the near future (Environmental Protection Agency 1993; World Meteorological Association 1995).

Looking for alternatives to methyl bromide has resulted in some successes and some failures (Chapman 1992; Linderman et al. 1994). Substituting methyl bromide with other biocide chemicals has worked in some situations, but has often not provided all the advantages of methyl bromide (James and Beall 1999; Kelpsas and Campbell 1994). However, in our evaluation at the Lone Peak Conservation Nursery, we found that 300 lbs./acre of chloropicrin alone was as effective in controlling soilborne pathogens as 350 lbs./acre of MBC. Chloropicrin not only reduced populations of potential pathogens in soil at least as much as MBC, but seedling density at the end of the growing season was similar. Seedling heights and diameters were larger in chloropicrin-treated

plots than in those treated with MBC. Therefore, by the parameters we used to evaluate soil fumigation efficacy, chloropicrin was at least equal to and in some cases superior to MBC in our trial.

Fallowing for at least one growing season, accompanied by periodic cultivation to keep weed populations low, helped reduce populations of potential pathogens in our trial. However, surviving populations were sufficient to adversely affect seedling production. Seedling density was reduced compared to either chloropicrin- or MBC-treated plots. Although seedling sizes (height, diameter, and root biomass production) in fallowed plots were similar to those in chloropicrin- and MBC-treated plots, this may be mostly due to enhanced seedling growth responding to reduced competition in lower density seedbeds.

As expected, the major group of pathogens found in the soil and isolated from diseased seedlings was *Fusarium*. Initially, the major species obtained from pretreated soil was *F. solani*. The proportion of this species within the *Fusarium* population declined following treatment, although it was still fairly prominent even at the end of the growing season. *Fusarium solani* was also commonly isolated from diseased bitterbrush seedlings. This species is a very common pathogen of a wide range of agricultural hosts and routinely induces root decay and deterioration (James and Perez 2000; Kendra and Hadwiger 1987; Li et al. 1998). It can survive in soil for prolonged periods due to its ability to form chlamydospores and is therefore difficult to eliminate from production soils (Burgess 1981; Cochrane and Cochrane 1966; Nelson et al. 1983). Another prominent *Fusarium* species in nursery soil was *F. oxysporum*. This species increased in response to soil treatments and was quite common in soil at the end of the growing season, although it was not isolated from diseased seedlings. *Fusarium oxysporum* is probably the most important *Fusarium* species limiting production of bare root conifer seedlings within western North America nurseries (James et al. 1991) and has been associated with seedling mortality previously at the Lone Peak Nursery (James 2002b). The other prominent *Fusarium* species encountered was *F. equiseti*, a species well-adapted to soil (Burgess 1981; Nelson et al. 1983). Populations of *F. equiseti* increased throughout the growing season and this species was routinely isolated from diseased seedlings.

Our field trial on bitterbrush seedlings provided the necessary information to recommend using chloropicrin as a viable alternative to MBC as a preplant soil fumigant. Chloropicrin is a very good fungicide, but has less efficacy killing many important weed seeds (Duniway 2002; Haar et al. 2003; Porter et al. 1999), although it may be more effective against weeds at higher application rates (Haar et al. 2003). An enhanced herbicide or hand weeding program may be necessary when substituting MBC with chloropicrin. Costs of application of MBC and chloropicrin are similar. Among the currently available alternatives to methyl bromide, chloropicrin has the fewest obstacles and is likely to be used extensively in the future (Haar et al. 2003). Chloropicrin does not deplete stratospheric ozone and degrades rapidly in the soil and atmosphere into environmentally benign products (Castro and Belser 1981; Gan et al. 2000).

We suspect that chloropicrin can be used as a satisfactory alternative to MBC at the Lone Peak Nursery, at least in bare root fields producing bitterbrush seedlings. It should work similarly with other hardwood or conifer species, although further efficacy tests may be warranted.

ACKNOWLEDGEMENTS

The authors thank Mike Marsden (USDA Forest Service) for help with statistical analyses and Brandon Long and Edie Trimmer (Lone Peak Conservation Nursery) for field assistance with the study.

LITERATURE CITED

Austin, D.D. and P.J. Urness. 1983. Summer use of bitterbrush rangelands by mule deer. *In*: Tiedemann, A.R. and K.L. Johnson (compilers). Proceedings – Research and Management of Bitterbrush and Cliffrose in Western North America. USDA Forest Service, Intermountain Forest and Range Experiment Station. General Technical Report INT-152. pp. 203-212.

Barnett, H.L. and B.B. Hunter. 1998. Illustrated genera of imperfect fungi. The American Phytopathological Society Press, St. Paul, MN. 218p.

Boone, A.J. 1988. Soil fumigation in forest tree nurseries. Proceedings of the Southern Forest Nursery Association – 1988. pp. 33-38.

Booth, C. 1966. The genus *Cylindrocarpon*. Commonwealth Mycological Institute. Kew, Surrey, England. Mycological Papers No. 104. 56p.

Boyd, R.J. 1971. Effects of soil fumigation on production of conifer nursery stock at two northern Rocky Mountain nurseries. USDA Forest Service, Intermountain Forest & Range Experiment Station. Research Paper INT-91. 19p.

Burgess, L.W. 1981. General ecology of the Fusaria. *In:* Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). *Fusarium:* Diseases, Biology & Taxonomy. The Pennsylvania State University Press, University Park. pp. 225-235.

Campbell, S.J. and B.R. Kelpsas. 1988. Comparison of three soil fumigants in a bareroot conifer nursery. Tree Planters' Notes 39(4):16-22.

Castro, C.E. and N.O. Belser. 1981. Photohydrolysis of methyl bromide and chloropicrin. Journal of Agricultural Food Chemistry 29:1005-1008.

Chapman, W. 1992. Alternative treatments to methyl bromide. Proceedings of the Southern Forest Nursery Association – 1992. pp. 96-103.

Cochrane, V.C. and J.C. Cochrane. 1966. Spore germination and carbon metabolism in *Fusarium solani*. V. Changes in anaerobic metabolism and related enzyme activities during development. Plant Physiology 41:810-814.

Cordell, C.E. 1982. Effective soil fumigation. *In*: Proceedings of the 1982 Southern Nursery Conference, Oklahoma City & Savannah, GA. USDA Forest Service, Southern Region. pp. 196-201.

Duniway, J.M. 2002. Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology 92:1337-1343.

Environmental Protection Agency. 1993. Regulation on methyl bromide. Federal Register 58:336 (December 10, 1993).

Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

Gan, J., S.R. Yates, F.F. Ernst and W.A. Jury. 2000. Degradation and volatilization of the fumigant chloropicrin after soil treatment. Journal of Environmental Quality 29:1391-1397.

Gandy, D.G. and D.O. Chanter. 1976. Some effects of time, temperature of treatment, and fumigant concentration on the fungicidal properties of methyl bromide. Annals of Applied Biology 82:279-290.

Harr, M.J., S. A. Fennimore, H. A. Ajwa and C.Q. Winterbottom. 2003. Chloropicrin effect on weed seed viability. Crop Protection 22:109-115.

Hamm, P.B. and E.M. Hansen. 1990. Soil fumigation, cover cropping, and organic soil amendments: their effect on soil-borne pathogens and the target seedlings. *In*: Rose, R., S.J. Campbell and T.D. Landis (eds.). Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations. USDA Forest Service, Rocky Mountain Experiment Station. General Technical Report RM-200. pp. 174-180.

Hansen, E.M., D.D. Myrold and P.B. Hamm. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. Phytopathology 80:698-704.

Hildebrand, D.M. and G.B. Dinkel. 1988. Evaluation of methyl bromide, Basamid granular, and solar heating for pre-plant pest control for fall-sown eastern redcedar at Bessey Nursery. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest, and Cooperative Forestry Management. Technical Report R2-41. 13p.

Hildebrand, D.M., J.K. Stone, R.L. James and S.J. Frankel. 2004. Alternatives to preplant soil fumigation for western forest nurseries. USDA Forest Service, Pacific Northwest Research Station. General Technical Report PNW-GTR-608. 27p.

Ibarbia, E.Z. 1995. Fundamentals of fumigation. Greenhouse Grower 13(1):141-142, 144.

James, R. L. 1982. Pythium root disease of Douglas-fir and grand fir seedlings at the Coeur d'Alene Nursery, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 82-10. 10p.

James, R. L. 1984. Biology and management of Botrytis blight. *In*: Murphy, P. M. (compiler). The Challenge of Producing Native Plants for the Intermountain Area. Proceedings: Intermountain Nurseryman's Association 1983 Conference. USDA Forest Service, Intermountain Forest and Range Experiment Station, General Technical Report INT-168. pp. 39-43.

James, R.L. 1989. Effects of fumigation on soil pathogens and beneficial microorganisms. *In*: Landis, T.D. (tech. coord.). Proceedings: Intermountain Forest Nursery Association Meeting. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report GTR-184. pp. 29-34.

James, R.L. 2002a. Investigations of potential disease-causing organisms associated with production of container-grown bitterbrush seedlings - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 146. 9p.

James, R.L. 2002b. Root disease of 1-0 bare root ponderosa pine seedlings – Lone Peak Nursery, Draper, Utah. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes #147. 8p

James, R.L. and K. Beall. 1999. An evaluation of the effects of dazomet on soil-borne diseases and conifer seedling production - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-9. 15p.

James, R.L. and K. Beall. 2000. Effects of fallowing on Fusarium-associated root diseases and production of bare root ponderosa pine seedlings at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-3. 13p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the First Meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

James, R.L., G.R. Knudsen and M.J. Morra. 2004a. Effects of preplant soil treatments on production of western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 04-12. 10p.

James, R.L., G.R. Knudsen and M.J. Morra. 2004b. Preplant soil treatment effects on production of Douglas-fir seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 04-10. 14p.

James, R.L., G.R. Knudsen and M.J. Morra. 2004c. Preplant soil treatment effects on production of ponderosa pine seedlings at the USDA Forest Service Lucky Peak Nursery, Boise Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 04-13. 12p.

James, R.L., S. Metzger and C.J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.

James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-6. 10p.

James, R.L. and R. Perez. 2000. Pathogenic characteristics of *Fusarium solani* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-15. 12p.

Kendra, D.F. and L.A. Hadwiger. 1987. Cell death and membrane leakage not associated with the induction of disease resistance in peas by chitosan or *Fusarium solani* f.sp. *phaseoli*. Phytopathology 77:100-106.

Kelpsas, B.R. and S.J. Campbell. 1994. Influence of mechanical incorporation method on dazomet distribution in conifer nursery soil. Tree Planters' Notes 45(2):53-57.

Kolbezen, M.J., D.E. Munnecke, W.D. Wilbur, L.H. Stolzy, F.J. Avu-El-Haj and T.E. Szuszkiewicz. 1974. Factors that affect deep penetration of field soils by methyl bromide. Hilgardia 42:465-492.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research (Japan) 8:114-125.

Knudsen, G.R. and L. Bin. 1990. Effects of temperature, soil moisture, and wheat bran on growth of *Trichoderma harzianum* from alginate pellets. Phytopathology 80:724-727.

Knudsen, G.R., D.J. Eschew, L.M. Dandurand and Z.G. Wang. 1991. Method to enhance growth and sporulation of palletized biocontrol fungi. Applied and Environmental Microbiology 57:2864-2867.

Li, S., G.L. Hartman and L.E. Gray. 1998. Chlamydospore formation, production, and nuclear status in *Fusarium solani* f.sp. *glycines* soybean sudden death syndrome-causing isolates. Mycologia 90:414-421.

Linderman, R., W. Dixon, S. Fraedrich and R.S. Smith, Jr. 1994. Alternatives to methyl bromide: assessment of research needs and priorities for forestry, nursery, and ornamental crops. Tree Planters' Notes 45:43-47.

Marois, J.J., M.T. Dunn and G.C. Papavizas. 1983. Reinvasion of fumigated soil by *Fusarium oxysporum* f.sp. *melonis.* Phytopathology 73:680-684.

Munnecke, D.E. and S.D. Van Gundy. 1979. Movement of fumigants in soil, dosage responses, and differential effects. Annual Review of Phytopathology 17:405-429.

Nelson, P.E. T.A. Toussoun and W.F.O. Marasas. 1983. Fusarium species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

Porter, I.J., R.W. Brett and R.M. Wisemen. 1999. Alternatives to methyl bromide: chemical fumigants or integrated pest management systems. Australasian Plant Pathology 28:65-71.

Stone, J.K., D.M. Hildebrand, R.L. James, S.M. Frankel and D.S. Germandt. 1995. Alternatives to methyl bromide for control of soil-borne diseases in bare root forest nurseries. *In*: Proceedings: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. November 6-8, 1995, San Diego, CA. Methyl Bromide Alternatives Outreach, US Environmental Protection Agency and US Department of Agriculture. pp. 77-1 – 77-4.

Shaheen, L. 1996. Potential loss of methyl bromide to prompt changes in Clean Air Act. Pest Control 64(5):68,74.

Smith, R.S., Jr. and R.V. Bega. 1966. Root disease control by fumigation in forest nurseries. Plant Disease Reporter 50:245-248.

Smolinska, U., M.J. Morra, G.R. Knudsen and R.L. James. 2003. Isothiocyanates produced by Brassicaceae species as inhibitors of *Fusarium oxysporum*. Plant Disease 87:407-412.

Stone, J.K., D.M. Hildebrand, R.L. James, S.M. Frankel and D.S. Germandt. 1995. Alternatives to methyl bromide for control of soil-borne diseases in bare root forest nurseries. *In*: Proceedings: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. November 6-8, 1995, San Diego, CA. Methyl Bromide Alternatives Outreach, US Environmental Protection Agency and US Department of Agriculture. pp. 77-1 – 77-4.

Vaartaja, O. 1967. Reinfestation of sterilized nursery seedbeds by fungi. Canadian Journal of Microbiology 13:771-776.

World Meterological Association. 1995. Scientific assessment of ozone depletion: 1994 executive summary. Global Ozone Research and Monitoring Project Report No. 37. Global Ozone Observing System, Geneva, Switzerland. 36p.

R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email rjames@fs.fed.us. S. Zeidler is former grower, Lone Peak Conservation Nursery, Draper, Utah 84020; email: scottzeidler@utah.gov.